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# Study of microbial proliferation and the *in vitro* antibacterial traits of commonly available flowers in Dhaka Metropolis

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## PEER REVIEW

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## Comments

The research introduced both the fundamental question of the usefulness of these readily applied flowers extracts and their potential as sources of microbial diseases if not prepared with caution. This is important since in some localities these plants are applied in their raw state.

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## ABSTRACT

**Objective:** To examine the prevalence of microorganisms and the antibacterial feature within commonly available flowers including *Hibiscus rosa-sinensis*, *Ixora coccinea*, *Ipomoea digitata*, *Allamanda cathartica*, *Nymphaea nouchali* and *Vinca rosea*, samples were randomly collected from different areas in Dhaka city, Bangladesh.

**Methods:** Conventional cultural and biochemical methods were applied to isolate and enumerate the flower accessing microorganisms. Flower extracts were prepared using the solvent extraction methods and the subsequent antibacterial activities were demonstrated.

**Results:** The total bacterial load and fungal load was estimated to be around  $10^7$ – $10^8$  CFU/g and  $10^5$ – $10^7$  CFU/g, respectively. All samples were found to be shaded with *Staphylococcus* spp. ( $\sim 10^7$  CFU/g) while the prevalence of actinomycetes was also observed except for *Ipomoea digitata*. The prevalence of Gram negative pathogenic bacteria was also noted within more than 50% samples. The *in vitro* antibacterial activity of these flowers, especially of *Ixora coccinea*, *Hibiscus rosa-sinensis*, *Allamanda cathartica* and *Nymphaea nouchali* in alcoholic extracts (methanol and ethanol) was notable against most of the tested microorganisms.

**Conclusions:** The contaminating microbial flora identified within the flower samples studied could be a potential environmental hazard if disseminated. Conversely the revealed antibacterial traits of the flower extracts would be useful alternate remedies of the synthetic drugs for disease medication.

## KEYWORDS

*Hibiscus rosa-sinensis*, *Ixora coccinea*, *Ipomoea digitata*, *Allamanda cathartica*, *Nymphaea nouchali*, *Vinca rosea*, Microorganisms, Antimicrobial activity, Public health

## 1. Introduction

Besides a mass usage of antibiotics to combat diseases, more than 50% of all drugs have been derived from natural sources around the globe till date<sup>[1–7]</sup>. A number of antibiotics have become almost archaic due to the emergence of resistant or even the multi-resistant strains, or have been reported to be associated with adverse side effects<sup>[8–10]</sup>. Commencing the herbal medication could be an alternative to treat diseases caused by multi-drug resistant bacteria<sup>[11,12]</sup>. Some other advantages with the usage of

herbal medicines also lie under the lesser side effects with small or no toxicity, cost effectiveness, better availability, etc<sup>[13–15]</sup>. Researchers are therefore increasingly turning their attention to herbal medicine to develop drugs with least or no side effects during medication<sup>[16–19]</sup>. In this context, flowers could be a potential part of microbial proliferation and conversely flower extracts might be of prospective health significance because of their ability to possess antibacterial attributes. While an array of plants has long been used for the preparation of herbal medicines, a significant fraction ( $\sim 10\%$ ) of flowering plants are also

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used for disease medication since they have been reported to possess the antioxidant activity with wound–healing effects[20–22]. Global usage of flowers for disease medication is thus not uncommon[16,23–26]. However, despite the fact that Bangladesh is a land of plenty of herbs and flowers, the microbial aspect or the medicinal usage of these flowers have not been well chalked out.

*Hibiscus rosa-sinensis* (*H. rosa-sinensis*), a commonly available flower in Bangladesh, are widely used in the treatment of a range of diseases including cardiac complications and the flower extracts are also known to possess the antibacterial activity against common pathogenic microorganisms[21,26]. *Ixora coccinea* (*I. coccinea*), another familiar flower not only in Bangladesh but also in India, Sri Lanka, Southeast Asia, and in the tropical areas, is extensively used as traditional medicine to mitigate common diseases including diarrhea, fever, sore, ulcers, skin diseases, and even cancer[27,28]. *Ipomoea digitata* (*I. digitata*), a flower locally named as Giant Potato, have been well known for its use to lower the blood pressure and for the treatment of poor digestion, tuberculosis and liver complications[5,29,30]. *Allamanda cathartica* (*A. cathartica*) is significantly renowned to exhibit activity against microorganisms and has been used for medication of fatal diseases including tumors, malaria, and liver complications too[24,25,31]. *Nymphaea nouchali* (*N. nouchali*), the national flower of Bangladesh, has been found to possess antidiabetic, anti-inflammatory, and antihepatotoxic activities[32,33]. *Vinca rosea* (*V. rosea*), another common flower in Bangladesh has the illustrious global usage to treat diabetes, high blood pressure, malaria, kidney disorder, skin infections and others[23,34].

Aside the effectiveness of flowers to possess the potential of exhibiting the antibacterial activity, a variety of bacteria and fungi has been reported to populate the floral nectar[35]. Knowledge on the microorganisms prevailing within flowers could fortify the hygiene consciousness and to trim down the infections acquired from environment[36]. Along these lines, in the present study we aimed to identify the flower contaminating harmful microorganisms as well as to assess the antibacterial activity of flower extracts of *H. rosa-sinensis*, *I. coccinea*, *I. digitata*, *A. cathartica*, *N. nouchali* and *V. rosea* extracts.

## 2. Materials and methods

### 2.1. Study area, sampling and sample processing

Samples of 6 categories of flowers including *I. coccinea*; *H. rosa-sinensis*; *I. digitata*; *A. cathartica*; *N. nouchali* and *V. rosea* were randomly collected during August 2013–October 2013 following standard protocol[37]. For identification and enumeration of flower contaminating bacteria and fungi, 10 g of each sample was homogenized with 90 mL buffer peptone (peptone, sodium chloride, disodium phosphate, mono-potassium phosphate) water (pH 7.2±0.2) and serially diluted

up to 10<sup>-5</sup>.

### 2.2. Microbiological analysis and confirmative biochemical tests

For each of the samples, 0.1 mL from the dilution 10<sup>-4</sup> was introduced on to the Nutrient agar (Hi-Media Laboratories Pvt. Ltd., India) and sabouraud dextrose agar (Hi-Media Laboratories Pvt. Ltd., India) for the isolation of total viable bacteria and fungi, respectively. Subsequently, different selective media such as MacConkey agar (Hi-Media Laboratories Pvt. Ltd., India), membrane fecal coliform agar (M-FC) (Hi-Media Laboratories Pvt. Ltd., India), mannitol salt agar (Merck Specialities Pvt. Ltd, Mumbai, India), cetrimide agar (Hi-Media Laboratories Pvt. Ltd., India) and actinomycetes agar (Oxoid Ltd., Basingstoke, Hampshire, England) were used for the determination of coliforms, fecal coliforms, *Staphylococcus* spp. *Pseudomonas* spp. and actinomycetes, consecutively[38]. All the inoculated plates were incubated at 37 °C for 24 h except sabouraud dextrose agar plates, which were incubated at 25 °C for 48 h. All the isolates were analyzed by a series of confirmative biochemical tests[38].

### 2.3. Processing of flower samples prior to the assay for the antibacterial activity

Three parts, *i. e.*, petals, steam and sepal from each of the flower samples were aseptically picked processed for the detection of antimicrobial activity. Fresh plant materials were washed with tap water and total 25 g of sample were homogenized with 75 mL of buffer peptone water to prepare the crude fraction.

#### 2.3.1. Aqueous extraction

Samples were dried thoroughly in the shade place, and blended to form a fine powder. A total of 10 g powder of each dried flower (petals, steam and sepal) were soaked in 140 mL of distilled water in Durham's bottle (Schott Duran, Germany) (*i. e.*, distilled water extract) and kept in shaking water bath (Daihan Scientific Co., Ltd, Korea, Model No-WSB-30) at 130 r/min for 24 h at 20 °C. Samples were aseptically filtered through sterile Whatman No 1 filter paper (Hangzhou Xinhua Paper Industry Co., Ltd., Hangzhou, China). Then the liquid portions were collected and the remaining samples were air dried to accelerate the next step of the experiment [39].

#### 2.3.2. Solvent extraction

Subsequently, 10 g of the dried flower powders of each component (petals, steam and sepal) were added with 90 mL of ethanol and methanol in Durham's bottle and were kept in shaking water bath (WSB-30, Korea) at 130 r/min for 24 h. at 20 °C. After filtration the liquid portion were collected and the remaining samples were air dried. Afterward, the dried residual extracts were dissolved in 10% dimethyl

sulfoxide (Merck Specialities Pvt Ltd Mumbai, India) to a final concentration of 10 mg/mL. Samples were stored overnight at  $-20^{\circ}\text{C}$ [26,39–41].

## 2.4. Antimicrobial assay

Modified agar well diffusion method was followed using Mueller–Hinton agar (MHA) plates (Oxoid Ltd., Basingstoke, Hampshire, England)[42,43]. The suspension of *Escherichia coli* (*E. coli*), *Pseudomonas* spp., *Salmonella* spp., *Listeria* spp., *Vibrio* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Bacillus* spp. were introduced on to the MHA media. Then wells (8 mm) were made on the inoculated MHA media and 100  $\mu\text{L}$  of the sample (crude fraction, aqueous, ethanol, methanol and residual extracts) at a concentration of 11.1 mg/mL were introduced. Absolute ethanol (Merck Specialities Pvt. Ltd, Germany), methanol (Merck Specialities Pvt. Ltd, Germany), buffer peptone water and dimethyl sulfoxide (10%) were applied as negative controls. Antibiotic disc of gentamicin (10  $\mu\text{g}$  (Oxoid Ltd., Basingstoke, Hampshire, England) was used as positive control. Plates were incubated at  $37^{\circ}\text{C}$  for 12–18 h, and were examined for the zone of inhibitions (mm).

## 3. Results

### 3.1. Prevalence of microorganisms in flower samples studied

Almost all samples have been found to be populated by a huge range of bacterial and fungal flora (Tables 1 and 2). No growth of *E. coli* was detected in *H. rosa-sinensis* and *V. rosea*. *Pseudomonas* spp. and *Klebsiella* spp. were only found in *I. coccinea* and *H. rosa-sinensis*. *Staphylococcus* spp. was the most prevalent among four types of bacteria. Actinomycetes were also present in almost all flower samples except *I. digitata*.

**Table 1**

Confirmative biochemical tests for the identification of specific microbial isolates.

Assumed pathogenic microorganisms	TSI			H <sub>2</sub> S	Indole	MR	VP	Citrate	Motility
	Slant	Butt	Gas	reaction	production	test	test	test	
<i>E. coli</i>	Y	Y	–	–	+	+	–	+	+
<i>Klebsiella</i> spp.	Y	Y	+	–	–	–	–	+	–
<i>Staphylococcus</i> spp.	Y	R	+	+	–	+	–	+	+
<i>Pseudomonas</i> spp.	R	R	–	–	–	–	–	+	+

TSI: triple sugar iron test; Y: yellow (acid); R: red (alkaline); MR: methyl red; VP: Voges–Proskauer.

**Table 2**

Microbiological condition of flower samples.

Test Samples (n=5)	TVB (CFU/g)	Fungi (CFU/g)	Actinomycetes (CFU/g)	<i>E. coli</i> (CFU/g)	<i>Klebsiella</i> spp. (CFU/g)	<i>Staphylococcus</i> spp. (CFU/g)	<i>Pseudomonas</i> spp. (CFU/g)
<i>I. coccinea</i> (Rangan)	$1.04 \times 10^8$	$1.40 \times 10^5$	$3.60 \times 10^4$	$2.76 \times 10^4$	$1.00 \times 10^4$	$1.76 \times 10^7$	$2.76 \times 10^4$
<i>H. rosa-sinensis</i> (Jaba)	$2.40 \times 10^8$	$5.04 \times 10^6$	$2.00 \times 10^5$	0	$1.30 \times 10^5$	$1.80 \times 10^7$	$1.50 \times 10^7$
<i>I. digitata</i> (Giant potato)	$3.55 \times 10^7$	$4.80 \times 10^7$	0	$4.10 \times 10^6$	0	$1.28 \times 10^7$	0
<i>A. cathartica</i> (Allamanda)	$5.84 \times 10^7$	$2.75 \times 10^7$	$2.17 \times 10^7$	$1.45 \times 10^7$	0	$1.36 \times 10^7$	0
<i>N. nouchali</i> (Water Lily)	$8.64 \times 10^7$	$1.28 \times 10^7$	$1.30 \times 10^6$	$2.82 \times 10^7$	0	$2.60 \times 10^7$	0
<i>V. rosea</i> (Nayantara)	$7.48 \times 10^7$	$2.40 \times 10^7$	$1.40 \times 10^7$	0	0	$2.32 \times 10^7$	0

TVB: total viable bacteria. The average microbial load has been shown in the table. *E. coli* and *Klebsiella* spp. expressed the coliform group. Fecal coliform, were completely absent in all samples. The experiment has been done in triplicate and the result was reproducible.

### 3.2. Antibacterial activity of flower extracts

The crude fraction of *H. rosa-sinensis* and *I. coccinea* showed no antimicrobial activity against any test organism (Tables 3 and 4). The fraction of *I. digitata* showed strongest activity against *Staphylococcus* spp. followed by other test bacteria except *Vibrio* spp., *Listeria* spp. and *Salmonella* spp. (Table 5). The crude fraction of *A. cathartica* showed activity against almost all test organisms except *Listeria* spp. The strongest activity was recorded against *Klebsiella* spp. and *E. coli*. (Table 6). The activity of *N. nouchali* was observed only against *Listeria* spp. (Table 7) while *V. rosea* showed the activity against *Klebsiella* spp. and *Listeria* spp. (Table 8). Notably the residual extracts (left after solvent extraction) of any flower samples possessed no antimicrobial activity against the test organism.

Among the distilled water washed raw flower blends (*i.e.*, aqueous extracts), no activity for *H. rosa-sinensis*, *N. nouchali* and *I. digitata* against any of the test microorganism was observed. Activity for *I. coccinea* was noted against *Staphylococcus* spp. (13 mm) while *V. rosea* was found to exhibit antibacterial activity against *Salmonella* spp. (11 mm) and *Klebsiella* spp. (8 mm). *A. cathartica* was found to exhibit the highest antibacterial activity against *Staphylococcus* spp. (30 mm), followed by *Bacillus* spp. (22 mm) and *Vibrio* spp. (18 mm). The activity against *Salmonella* spp. was for *A. cathartica* was also observed (10 mm).

### 3.3. Ethanol extracts

The ethanol extracts of *H. rosa-sinensis* and *N. nouchali* showed antimicrobial activity against almost all test organisms except *Vibrio* spp. (Tables 3 and 7). Ethanol extracts of *I. coccinea* and *A. cathartica* showed activity against all the test organisms (Tables 4 and 6). *I. digitata* showed the strongest activity against *Bacillus* spp. followed by *Klebsiella* spp., *Staphylococcus* spp., *Salmonella* spp., and *Pseudomonas* spp. and *E. coli*. No activity against *Vibrio* spp. and *Listeria* spp. was observed (Table 5). In *V. rosea* the highest activity was observed against *Salmonella* spp. and *Pseudomonas* spp. while the activity against *E. coli*, *Staphylococcus* spp., *Vibrio* spp. and *Bacillus* spp. was found to be relatively low. No antimicrobial activity was scored against *Klebsiella* spp. and *Listeria* spp. (Table 8).

### 3.4. Methanol extracts

The methanol extract of *H. rosa-sinensis*, *I. coccinea*, *A.*

**Table 3**Antimicrobial activity of *H. rosa-sinensis* (Jaba).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	0	0	0.00	19.72	0.00	14.81	16.80
<i>Pseudomonas</i> spp.	0	0	0.00	18.00	0.00	11.60	39.87
<i>Vibrio</i> spp.	0	0	3.38	0.00	9.75	11.69	18.01
<i>Bacillus</i> spp.	0	0	0.00	11.12	0.00	12.60	21.09
<i>Klebsiella</i> spp.	0	0	21.80	22.81	0.00	12.38	18.83
<i>Staphylococcus</i> spp.	0	0	0.00	19.87	7.03	8.89	34.64
<i>Listeria</i> spp.	0	0	0.00	10.00	0.00	0.00	23.00
<i>Salmonella</i> spp.	0	0	19.92	30.49	0.00	0.00	29.37

**Table 4**Antimicrobial activity of *I. coccinea* (Rangan).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	0	0	13.72	16.28	0.00	13.02	16.80
<i>Pseudomonas</i> spp.	0	0	0.00	9.11	12.27	16.62	39.87
<i>Vibrio</i> spp.	0	0	3.38	7.37	9.75	10.41	18.01
<i>Bacillus</i> spp.	0	0	0.00	7.50	11.37	23.05	21.09
<i>Klebsiella</i> spp.	0	0	9.50	9.28	13.19	13.53	18.83
<i>Staphylococcus</i> spp.	0	0	0.00	21.07	7.03	10.95	34.64
<i>Listeria</i> spp.	0	0	0.00	9.50	10.98	11.02	23.00
<i>Salmonella</i> spp.	0	0	19.92	24.74	0.00	0.00	29.37

**Table 5**Antimicrobial activity of *I. digitata* (Giant Potato).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	10.80	0	15.27	18.02	0	7.49	25.51
<i>Pseudomonas</i> spp.	11.40	0	0.00	24.46	0	9.07	29.04
<i>Vibrio</i> spp.	0.00	0	0.00	0.00	0	0.00	18.01
<i>Bacillus</i> spp.	12.09	0	24.51	30.72	0	0.00	38.80
<i>Klebsiella</i> spp.	11.78	0	23.12	27.92	0	0.00	18.83
<i>Staphylococcus</i> spp.	12.46	0	0.00	26.67	0	0.00	34.96
<i>Listeria</i> spp.	0.00	0	21.41	0.00	0	8.18	28.57
<i>Salmonella</i> spp.	0.00	0	0.00	25.43	0	0.00	30.05

**Table 6**Antimicrobial activity of *A. cathartica* (Allamanda).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	15.36	0	15.27	18.00	0	13.26	25.51
<i>Pseudomonas</i> spp.	12.78	0	0.00	28.00	0	9.91	29.04
<i>Vibrio</i> spp.	14.30	0	0.00	21.00	0	17.00	20.00
<i>Bacillus</i> spp.	16.15	0	24.51	30.09	0	14.00	38.80
<i>Klebsiella</i> spp.	15.69	0	23.12	26.43	0	9.61	18.83
<i>Staphylococcus</i> spp.	11.97	0	0.00	18.00	0	16.00	34.96
<i>Listeria</i> spp.	0.00	0	21.41	26.23	0	11.00	28.57
<i>Salmonella</i> spp.	10.46	0	0.00	23.68	0	0.00	30.05

**Table 7**Antimicrobial activity of *N. nouchali* (Water Lily).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	0.00	0	13.34	16.55	0	7.49	25.06
<i>Pseudomonas</i> spp.	0.00	0	18.40	21.56	0	9.68	28.44
<i>Vibrio</i> spp.	0.00	0	0.00	0.00	0	10.25	20.00
<i>Bacillus</i> spp.	0.00	0	0.00	12.07	0	15.65	37.17
<i>Klebsiella</i> spp.	0.00	0	0.00	29.78	0	12.56	18.83
<i>Staphylococcus</i> spp.	0.00	0	0.00	21.84	0	0.00	32.36
<i>Listeria</i> spp.	12.94	0	0.00	19.90	0	9.19	26.79
<i>Salmonella</i> spp.	0.00	0	17.83	27.75	0	0.00	29.22

*cathartica* and *N. nouchali* showed antimicrobial activity against almost all test microorganisms (Tables 3, 4, 6 and 7). However, no antimicrobial activity of the methanol extract of *H. rosa-sinensis* was observed against *Listeria* spp. (Table 3). Moreover, none of the samples showed

antimicrobial activity against *Salmonella* spp. Extracts of *I. digitata* showed antimicrobial activity against *E. coli*, *Pseudomonas* spp., and *Listeria* spp. (Table 5). The activity of *V. rosea* extract was observed only against *Vibrio* spp. (Table 8).

**Table 8**Antimicrobial activity of *V. rosea* (Nayantara).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin)
<i>E. coli</i>	0.00	0	11.00	13.83	0	0.00	25.06
<i>Pseudomonas</i> spp.	0.00	0	18.40	22.40	0	0.00	28.44
<i>Vibrio</i> spp.	0.00	0	0.00	12.00	0	7.40	20.00
<i>Bacillus</i> spp.	0.00	0	11.00	10.00	0	0.00	37.17
<i>Klebsiella</i> spp.	12.08	0	0.00	0.00	0	0.00	18.83
<i>Staphylococcus</i> spp.	0.00	0	0.00	13.00	0	0.00	32.36
<i>Listeria</i> spp.	12.06	0	0.00	0.00	0	0.00	26.79
<i>Salmonella</i> spp.	0.00	0	17.83	24.29	0	0.00	29.22

## 4. Discussion

Earlier studies have explored the culture-independent diversity of yeasts and fungi on flowers or in nectar<sup>[44]</sup>. In the present study, total viable bacteria, fungi and *Staphylococcus* spp. were found in each of the flower samples studied. The huge proliferation of microorganisms in the samples studied here are in cohort with other recent studies where the nectar-dwelling microorganisms, particularly yeasts, have been shown to prevail in the animal-pollinated flowers<sup>[36,44,45]</sup>.

Several earlier studies noticed elevated levels of bacteria ( $10^4$ – $10^{10}$  CFU/mL), including Gram-negative bacteria and *Pseudomonas aeruginosa* in flowers<sup>[36,46–48]</sup>. However, analysis of resident microflora in flowers is indeed not that frequent especially in context of Bangladesh or other developing countries. The colossal propagation of microorganisms in the flower samples investigated in the current study may reflect the possibility of disease outbreaks<sup>[46]</sup>.

A large number of reports revealed the presence of enzymatic and non-enzymatic antioxidants like phenols, flavonoids, triterpenoids, umbelliferone, etc. which might in turn, impart the antibacterial activity<sup>[49,50]</sup>. While the medicinal plants are numerous in Bangladesh, and the root or leaf extracts are widely used to treat a variety of clinical complications, the study of flower extracts both in terms their antimicrobial traits still remains diminutive in local perspective<sup>[51]</sup>. So far huge reports on the antibacterial activity of the flower extracts exist. *H. rosa-sinensis* has been shown earlier to exhibit such effect in *E. coli*, *Bacillus* spp. and *Salmonella* spp.<sup>[26,52,53]</sup>. Ethanolic and aqueous root extracts of *I. coccinea* were found to inhibit the growth of several Gram negative and Gram positive bacteria but were inactive against fungi<sup>[27,28,54,55]</sup>. The methanol extracts of *N. nouchali* were found to exhibit antibacterial and antifungal activity against *Bacillus* spp., *Sarcina lutea*, *Shigella* spp., *Vibrio* spp. and *Saccharomyces cerevisiae*<sup>[56]</sup>; *A. cathartica* and *V. rosea* against numerous human pathogens<sup>[24,25]</sup>. The tuberous root powder extract of *I. digitata* showed significant antibacterial activity against *Pseudomonas aeruginosa* and *E. coli*<sup>[57]</sup>. Being led by the global colossal information on such traits of flowers, our study further turned to the demonstration of the antibacterial activity of the commonly available flower samples.

According to our observation, most of the extracts showed antibacterial activity against *E. coli*, *Bacillus*

spp., *Pseudomonas* spp., *Staphylococcus* spp., *Vibrio* spp., *Listeria* spp., *Klebsiella* spp. and *Salmonella* spp. However, the ethanol extracts showed the highest zone of inhibition against all pathogens rather than the methanol extracts, which is indeed consistent to several other studies<sup>[58]</sup>. Thus the ethanol and methanol extracts of the flowers are proposed to possess broad inhibitory activities to pathogenic microorganisms in our condition and hence are promising to act as potential antibacterial agents from natural plant sources. Such activity could be due to the presence of some active compounds in the flowers as noted earlier<sup>[26]</sup>.

The results of the antibacterial traits of the samples studied in the current investigation justifies the claimed uses of flowers in the traditional system of medicine to treat various infectious disease caused by the microbes. However, prior to usage, the associated toxicological study and subsequent clinical trials in animal model should be carried out. However, further studies are needed to better evaluate the potential effectiveness of the extracts as the antimicrobial agents. Overall, the findings of the current investigation may largely aid to the disease medication approach by using the flower extract besides the herbs not only of ours but also would be implementable in other developing countries.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

Several plants and plant products are medicines in several countries including India. Therapeutic effects have been attributed to majority of these plant derivatives including the flowers. The authors seek to confirm these associated antimicrobial activities of these plants in their natural state



in contrast to the ability of these microbes to grow and populate on these same plants.

### Research frontiers

Most herbal applications target the roots and the leaves. The idea of looking at the flower is an important alternative. Additionally, quantification of microorganism and their antibiotic sensitivity will reveal their suitability for the proposed therapeutic application.

### Related reports

The possibility of environmental basis of contaminating microorganism cannot be ruled out and will have been better if sampling was done in different regions of Dhaka to rule that out. However, the results are similar to results seen in most antimicrobial sensitivity assays with conventional and non-conventional antibiotics.

### Innovations & breakthroughs

The varied microbiological techniques applied, the different extraction methods and antimicrobial assays helped to bring out concrete results and arguments.

### Applications

Although no new methods were applied, the results will not only open up a discussion on the effectiveness of supposed therapeutic activity of these plant extracts but also the need to look at their processing to prevent any infection as a result of bacterial contamination.

### Peer review

The research introduced both the fundamental question of the usefulness of these readily applied flowers extracts and their potential as sources of microbial diseases if not prepared with caution. This is important since in some localities these plants are applied in their raw state.

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